

Supplemental material.

Surgical procedure for uterine perfusion. Pregnant rats on GD 18.5 were used for the uterine perfusion. A schematic illustration of uterine perfusion is shown in Figure 1A. After anesthesia by pentobarbital, the abdomen of the dam was opened and the uterine, abdominal aorta, caudal vena cava, uterine arteries, uterine veins, renal arteries, renal veins, and internal iliac artery and vein were exposed. All exposed tissues were moistened with saline buffer maintained at 37°C. Heparin was injected into the jugular vein. Except for the right uterine artery and vein, all blood vessels forked from the abdominal aorta and caudal vena cava were bound tightly at the fork from these vessels. Moreover, the internal iliac artery and vein were also bound. The right oviduct and the caudal side of the uterus of the target fetus, which was located on the most cranial side of right uterus, were bound tightly with blood vessels distributed. After oxygenated buffer solution was pumped in, the abdominal aorta was incised and cannulated with PE-10 polyethylene tube (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) to supply perfusate. A venular indwelling needle (Terumo, Tokyo, Japan) was inserted into the caudal vena cava as a drain tube. The abdominal aorta and caudal vena cava were bound at the cranial and caudal sites of tube insertion, respectively. In this system, the perfusate pumped into the abdominal aorta is circulated through the one unit of uterine artery–placenta–fetus–uterine vein, and dripped from the drain tube inserted in the caudal vein. After the perfusion, dams and fetus were euthanized by incision of caudal vena cava under the anesthesia condition and tissues were collected.

Primers. Sequences of oligonucleotide primers to amplify gene-specific cDNAs were as follows.

β -glucuronidase-Forward(F):5'-CCCTCCCTGTCGGGATTCTGA-3',
 β -glucuronidase-Reverse (R): 5'-CCACACCGGGACACTCATCG-3',
Mrp1-F: 5'-TCGAATGTCCTCTGAGATGGAGAC-3',
Mrp1-R: 5'-GGAAGTCTACACGGCCTGAATG-3',
Mrp2-F: 5'-AGGAACTGGAAGACCTTCATGAAGC-3',
Mrp2-R: 5'-CAGTCTACGGCGAAGACTATTTTC-3',
Mrp3-F: 5'-GCTATCCGACCTGGAGTCTAATATC-3',
Mrp3-R: 5'-AGCACGGTTGCTCTCCAACAC-3',
Oatp1a1-F: 5'-TTAGAAATGGGGAAGGTTGCTG-3',
Oatp1a1-R: 5'-TGGTTAATCCAGCAACTGCTGC-3',
Oatp1a4-F: 5'-CAGGAATGACCATTGGCCCTTTG-3',
Oatp1a4-R: 5'-AAGGTACTCAGACAGGCAGAGCCAG-3',
Oatp1a5-F: 5'-CGCTTGGGATTGGATTACATGC-3',
Oatp1a5-R: 5'-ATGAGACAGTGGCCTTTGGAGA-3',
Oatp1b2-F: 5'-CTAAGGTCTCTGAGAAGCATCC-3',
Oatp1b2-R: 5'-AACTGAAACGCATAGACCACTGAG-3',
Oatp4a1-F: 5'-GAGAGCAGCCGAAACACAGC-3',
Oatp4a1-R: 5'-CAGTAGACTGGTCAAGGTCC-3'.
UGT1A1-F: 5'-TGGTGTGCCGGAGCTCATGTTTCG-3'
UGT1A1-R: 5'-CTGCTGAATAACTCCGAGCATACT-3'
UGT1A6-F: 5'-CCTCAGTGAACGCGGACACGAC-3'
UGT1A6-R: 5'-TTCCTGTACTCTCTTAGAGGAGCCA-3'
UGT1A7-F: 5'-CAGTTGGCAGCTGGGAAAACCA-3'
UGT1A7-R: 5'-GAAGAAACCCTGGGAAGGGCTA-3'
UGT2B1-F: 5'-AGATGATGGGAAGGCAGAT-3'

UGT2B1-R: 5'-GCAAGAGCAGAAGCAACTAC-3'

GAPDH-F: 5'-TTCAACGGCACAGTCAAG-3',

GAPDH-R: 5'-CACACCCATCACAAACAT-3'.

All PCR products were sequenced by a Model 310 sequencer (Applied Biosystems, Foster City, CA, USA).

Immunohistochemical analysis. Placentae were collected from GD 18.5 dams and fixed in 4% paraformaldehyde/PBS, pH 7.0. After dehydration in serial concentrations of ethanol and xylene, fixed samples were embedded in paraffin and sectioned into 7- μ m-thick slices.

Following deparaffinization and hydration, the sections were immersed in 10 mM of sodium-citric acid buffer and microwaved for 15 min. After cooling to room temperature, 10% NGS/PBS was applied to the sections which were then incubated for 1 h at room temperature. Anti-Mrp1 (1:100, diluted with 10% NGS) or anti-Oatp4a1 antibodies (1:500, diluted with 10% NGS) were applied to the sections which were then incubated overnight at 4°C. For the controls, only 10% NGS was applied to the sections for overnight incubation at 4°C. After three washes with PBS containing 0.05% Tween 20, the specimens were incubated with fluorescein isothiocyanate (FITC) goat anti-rabbit IgG (H+L)-conjugate (1:100, diluted with PBS, ZyMax™ Grade, Zymed Laboratories Inc., S. San Francisco, CA, USA) and propidium iodide (PI, 1:200 dilution, Sigma-Aldrich, St. Louis, MO, USA) for 2 h at room temperature. Sections were mounted using Vector Shield (Vector Laboratories Inc., Burlingame, CA, USA) and then examined using a confocal laser scanning microscope (Axionvert 200M) and PASCAL software (Carl Zeiss Microimaging, Jena, Germany).

UGT enzyme analysis

Briefly, livers were collected from dams and fetus on GD18.5. The hepatic microsomes were activated with a final concentration of 0.05% sodium cholate. Then, microsomes were incubated with BPA or 1-NA, and 3mM of UDP-glucuronic acid at 37 °C for 30 min. After the reaction, mixture were boiled for 5 min and centrifuged at 13000g for 5 min. The supernatant was eluted by HPLC according to the analytical condition described in materials and methods.